

## Opinion

## HIV Reactivation after Partial Protection by Neutralizing Antibodies

Matthew S. Parsons,<sup>1,\*</sup> Deborah Cromer,<sup>2</sup> Miles P. Davenport,<sup>2</sup> and Stephen J. Kent<sup>1,3,4,\*</sup>

It is widely thought that generating broadly neutralizing anti-HIV antibodies (BnAbs) will protect humans against HIV, given promising data from *in vitro* experiments and *in vivo* macaque studies. The primary action of BnAbs is preventing cell-free virus from entering cells. Recent *in vitro* and macaque data suggest that BnAbs are less potent against cell-associated virus exposure. We speculate that BnAb-based suppression of HIV transmission, particularly if mediated by cell–cell transmission, may result in some exposed subjects carrying a form of latent (or ‘occult’) HIV infection. Such largely hidden HIV infections may subsequently reactivate when BnAb levels decline. This concept has implications for the achievement of long-term sterilizing immunity to HIV.

## Potential Limits of Neutralizing Antibody Immunity to HIV

A reproducibly efficacious approach for prevention of **simian/HIV (SHIV)** (see [Glossary](#)) infections in macaque models is the passive administration of **BnAbs** [1–3]. Two large human efficacy trials of passive administration of a monoclonal BnAb called VRC01 are currently ongoing (<https://clinicaltrials.gov>; NCT02568215 and NCT02716675). Larger-scale production of monoclonal antibodies for **passive transfer** and/or delivery of antibodies from gene therapy vectors may be one path towards protection of humans from **HIV** [4]. The monoclonal BnAbs currently studied for protective efficacy have largely been isolated from subjects with longstanding HIV infection. At present it is difficult to induce BnAbs by vaccination as extensive affinity maturation and somatic hypermutation of the antibodies is usually required to acquire substantial breadth and potency of neutralization. Novel vaccination strategies that rely on the stepwise mobilization of the correct germline precursors, followed by the induction of their maturation towards potent BnAb activity, are starting to show promise in human immunoglobulin loci transgenic mice [5].

There are, we postulate, important caveats to the widely accepted proposition that BnAbs will reliably protect against HIV. Most *in vitro*, murine, and macaque studies have examined the protective capacity of BnAbs against **cell-free virus** exposure. HIV is also present in infectious fluids in cell-associated forms that have long been speculated to provide a mechanism to evade HIV-specific immunity [6,7]. **Cell-associated HIV** is infectious in animal models [8,9] and one small study suggested that a subset of human transmissions was initiated by cell-associated HIV variants in the infected partners’ semen [10]. Many BnAbs have a reduced capacity to prevent cell-associated HIV transmission *in vitro* [11–13]. Our recent work in macaques showed that, compared with the standard cell-free challenge system, the BnAb PGT121 was only partially effective in preventing a high-dose cell-associated SHIV challenge [14].

## Highlights

BnAbs can efficiently protect against free HIV virions *in vitro* and in animal models.

The effectiveness of BnAbs is more limited against HIV within cells and transmission of HIV between cells.

Latent HIV in cells may reactivate when BnAb levels decline; this may occur weeks or years later.

Late recrudescence HIV infections may undermine the protective capacity of BnAbs.

<sup>1</sup>Department of Microbiology and Immunology, The University of Melbourne, The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

<sup>2</sup>Infection Analytics Program, Kirby Institute for Infection and Immunity, University of New South Wales Australia, Sydney, Australia

<sup>3</sup>Melbourne Sexual Health Centre and Department of Infectious Diseases, Alfred Health, Central Clinical School, Monash University, Melbourne, Australia

<sup>4</sup>ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Parkville, Australia

\*Correspondence: [mattp@unimelb.edu.au](mailto:mattp@unimelb.edu.au) (M.S. Parsons) and [skent@unimelb.edu.au](mailto:skent@unimelb.edu.au) (S.J. Kent).

BnAbs primarily inhibit free virus via neutralization, but a potential mechanism for antibodies to control cell-associated HIV is via Fc–Fc receptor interactions [15,16]. Even in the case of cell-free virus exposure, the Fc functions of BnAbs assist in mediating protection in both mouse and macaque models [17,18], presumably through antibody-mediated clearance [e.g., antibody-dependent cellular cytotoxicity (ADCC)] of limited numbers of cells that become infected despite the potent neutralization function of the BnAb. Many, but not all, BnAbs currently being studied have the capacity to mediate ADCC [19,20]. Fc–Fc receptor interactions are likely to be even more important in the clearance of cell-associated HIV. Fc–Fc receptor interactions do not require autologous cell–cell interactions (e.g., T cell receptor and MHC class I interactions) and thus they can recognize and eliminate allogeneic HIV Env-expressing cells [21].

A caveat with the capacity of Fc–Fc receptor interactions to clear infected cells is that infected cells downregulate CD4 and the expression of many Env-specific ADCC epitopes is dependent on an open form of the Env trimer following interactions between CD4 and Env [22,23]. BnAbs, however, recognize Env epitopes present on the closed trimer not dependent on CD4 and, if they can mediate ADCC, should be able to kill infected cells [19]. Antibodies that mediate ADCC but are not neutralizing may be only partially effective since they may select for ADCC escape mutations [24,25]. The ADCC capacity of BnAbs is linked to their ability to protect macaques from cell-free SHIV challenge [17].

### The Problem of Cells with Latent HIV Infection Despite Immunity

BnAbs that mediate ADCC will not, however, recognize and kill HIV-infected cells that express minimal or no **Env protein** on their surface [26,27]. Antiretroviral treatment of established HIV infection eliminates most cells expressing viral antigens but leaves a population of latently infected cells relatively invisible to immune responses. Reactivation of latently infected cells can result in an effective BnAb-mediated decrease of the **viral reservoir** in mouse models [28].

How latently infected cells are generated during established HIV infection is imperfectly understood, even in the setting of chronic ART administration where new infections are blocked. Post- and pre-exposure prophylaxis (PEP and PrEP) with antiretroviral therapy (ART) can result in infections that are not completely prevented but are suppressed until ART is ceased. The capacity of HIV or SHIV to establish occult infections that remain hidden until the waning of antiretroviral PrEP has now been noted in humanized mice and macaques [29,30]. In macaque simian immunodeficiency virus (SIV) studies where ART is started just a few days after SIV exposure, ART suppresses but does not eliminate infection, which recrudesces after ART is withdrawn [31]. Anecdotal evidence of a similar phenomenon has now been reported in a case study of human HIV infection while on PrEP [32]. A 50-year-old man became infected with non-drug-resistant HIV despite being PrEP adherent. This individual exhibited an altered seroconversion pattern, exhibiting anti-gp160 antibodies before developing anti-p24 antibodies. Furthermore, plasma viremia and cell-associated viral RNA and DNA were undetectable at the time of antibody detection. Plasma viremia was not detected until 3 weeks after cessation of PrEP. Given the evidence from animal studies and a case study of human HIV transmission during PrEP, large clinical trials evaluating the efficacy of PrEP should incorporate long-term follow-up protocols to evaluate the prevalence of HIV infection in participants after permanent discontinuation of PrEP.

In a vaccination setting, whether small numbers of latently infected cells form after a partially suppressed initial infection is difficult to study. Unvaccinated macaques serially exposed to low doses of SIV have been reported as having had what is termed an occult infection with intermittent detection of low levels of virus before an active infection ensuing much later [33]. In what form the

### Glossary

#### Broadly neutralizing antibodies to HIV (BnAbs):

neutralize most HIV strains and are typically protective in passive transfer studies in animal models.

**Cell-associated HIV:** HIV in cells, potentially hidden from BnAbs and other immune responses.

**Cell-free HIV:** individual virions; most animal model challenge studies use cell-free virus.

**Env protein:** the surface protein of HIV, existing as a closed trimer on HIV virions.

#### Half-life of fully protective BnAb immunity:

we define this as a threshold where BnAb levels effectively neutralize any reactivation of latent HIV.

#### HIV-exposed but uninfected subjects:

subjects repeatedly exposed to HIV but without detectable HIV virus or HIV antibodies. A subset of these people will have either mutations in CCR5, meaning that are naturally protected from HIV, or HIV-specific CTL responses, which have been postulated to protect them from future exposures.

**HIV reservoir:** the pool of latent HIV.

#### Human immunodeficiency virus (HIV):

worldwide, most infections occur with HIV-1. HIV-1 strains are highly diverse.

**Latent HIV:** HIV existing as integrated DNA in cells – usually resting CD4 T cells – without replicating. HIV expresses few if any HIV proteins in this state and is largely hidden from immune responses.

**Passive transfer:** administration of protective antibodies, usually intravenously. Passive transfer trials to assess the efficacy of the BnAb VRC01 are currently ongoing for HIV.

**Reactivation rate:** how frequently HIV reactivates to replicate. In chronic HIV treated with ART it is around once per week, but it is much slower when the reservoir of latent HIV is small.

**Recrudescence HIV:** latent HIV that reactivates to replicate.

**Simian/HIV (SHIV):** SIV modified to express the envelope of HIV isolates. These chimeric viruses are valuable tools for assessing the protective efficacy of antibodies directed

infection is present, and where the infection resides, is unknown. The infection is potentially in some form of latency in tissues with minimal expression of viral proteins.

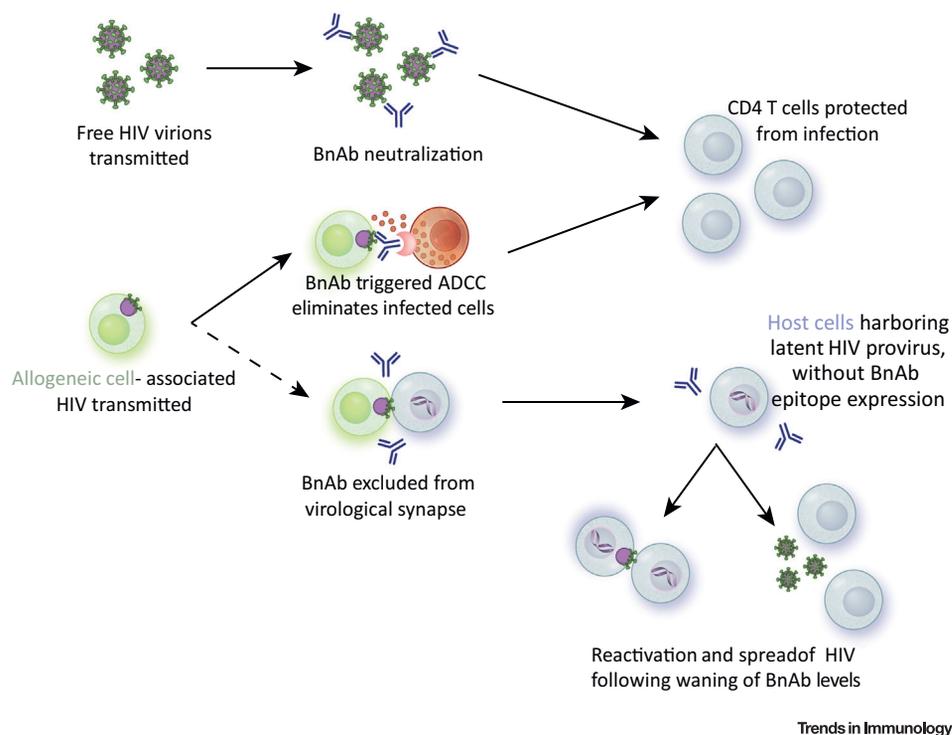
against the HIV envelope in nonhuman primates.

We recently observed such an event when a macaque administered the BnAb PGT121 and then challenged intravenously with a high dose (~1000 animal infectious doses) of cell-associated SHIV<sub>SF162P3</sub> had no detectable SHIV<sub>SF162P3</sub> RNA, cellular DNA, or seroconversion and exhibited **recrudescence** at 6–8 weeks [14]. Transfer of 22 million peripheral blood mononuclear cells (PBMCs) from this animal obtained during weeks 1–4 after SHIV<sub>SF162P3</sub> exposure to uninfected macaques – a previously reported sensitive measure for transferring infection [34] – failed to initiate an infection. This suggests that the levels of initial infection were very low and confined to tissues. The virus that eventually emerged remained neutralization sensitive and comprised only a single founder, suggesting that a low level of initial infection occurred that lay dormant. The timing of the recrudescence coincided with the decline in the passively transferred BnAb to low levels, suggesting the BnAb may have suppressed active infection but failed to clear all of the infected cells. Interestingly, cell-associated viral RNA and DNA became detectable in PBMCs 6 weeks post-challenge, just before the presence of viremia and seroconversion at week 8 post-challenge. The initial observation of cell-associated virus before viremia is similar to that reported in recrudescence after early ART in an infected infant (the ‘Mississippi baby’) [35]. Whether this phenomenon, observed in macaques exposed to a high-dose cell-associated SHIV<sub>SF162P3</sub> challenge, will occur in humans exposed to lower doses of cell-associated HIV is unclear at present. Allogeneic cells containing virus will of course be HLA-I mismatched, recognized as foreign, and rapidly eliminated. The ability of allogeneic cells to rapidly transfer virus to host lymphocytes, however, could result in autologous infected cells at various stages of viral replication (i.e., actively infected, pre-integration latency, and post-integration latency). Infected, but quiescent, autologous cells would be optimal candidates for the establishment of an occult infection able to propagate following BnAb waning.

Whether the cell-associated SHIV challenge employed above, compared with standard cell-free challenge models, made it more likely that a latent cell-associated SHIV infection lay dormant for some time is unclear. Liu *et al.* (2016) showed that even with PGT121 infusion, macaques challenged vaginally with cell-free virus exhibit limited viral replication at distal sites before viral elimination [36]. Given that BnAbs incompletely neutralize cell-to-cell transmission events [11], we hypothesize that exposure to cell-associated virus would increase the burden of replicative virus at distal sites requiring elimination by BnAbs to achieve immunity. There are also several possible models where cell–cell transmission could evade the neutralization and/or ADCC function of BnAbs (Figure 1). Waning suboptimal levels of BnAbs could contribute to such phenomena.

### When Would Latent HIV Infections Emerge after BnAb Levels Wane?

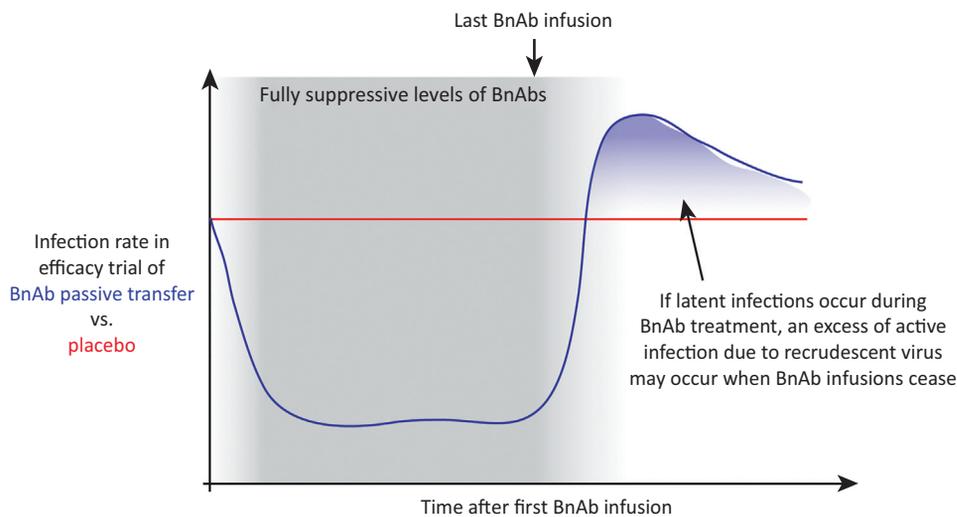
The RV144 trial results show that prevention of HIV by vaccination is likely to be achievable [37]. It seems likely that the VRC01 passive BnAb transfer human efficacy trials will provide some protection from infection during the 80 weeks when the infusions are given. Given the results of our macaque study and human observations noted above, it is possible that some infections may be initiated during the time of the BnAb infusions that emerge at some point after the infusions cease. It will be relatively difficult to know when such infections are acquired in the absence of clear epidemiological evidence of transmission. If significant numbers of suppressed infections are acquired during BnAb therapy, we speculate that there may be a higher rate of apparent infections early after the infusions end in the BnAb group than in the control group (Figure 2).



**Figure 1. Illustrations of HIV Infection Scenarios and Impact of Broadly Neutralizing Anti-HIV Antibodies (BnAbs).** BnAbs are protective against cell-free virus. They also provide some protection from cell-associated HIV through antibody-dependent cellular cytotoxicity (ADCC). However, in some cases cell-associated HIV may be transmitted to host cells through a virological synapse that excludes BnAb. If infected host cells are latently infected, they remain hidden from BnAbs and may reactivate to initiate a spreading infection when BnAb levels wane.

The timing of reactivation of an established latent infection after ART is ceased has been studied intensively as it is an important end point in HIV cure-related research [38]. How such observations will translate to reactivation of infections suppressed by passive or active vaccination approaches is less clear. Reactivation of latent virus begins around 7 days after ART is ceased [38]. However, the cessation of BnAb infusions results in a much longer period of subtherapeutic levels of the BnAb, probably at several weeks with current versions and potentially much longer with longer-acting BnAbs under development. A recent passive transfer study in macaques showed that a single infusion of more potent and longer-acting BnAbs was protective against serial low-dose SHIV challenges for up to 23 weeks [39]. In the setting of active vaccination, although not achieved to date, vaccine-induced BnAb levels could have a long subtherapeutic tail of many years.

We can model how reactivation events of BnAb-suppressed **latent HIV** infections might occur as the level of protection afforded by BnAbs wanes over time. The model assumes that, above a certain threshold level, BnAbs fully protect against any reactivating infections. However, once BnAb levels wane their protection is only partially efficacious and the antibodies still allow a proportion of reactivating infections to grow, albeit at a slower rate than 'normal'. The model assumes that the protection afforded by BnAbs is proportional to the level of antibody present and that latent infections reactivate according to a Poisson process. Full details of the model are given in the supplemental information online.

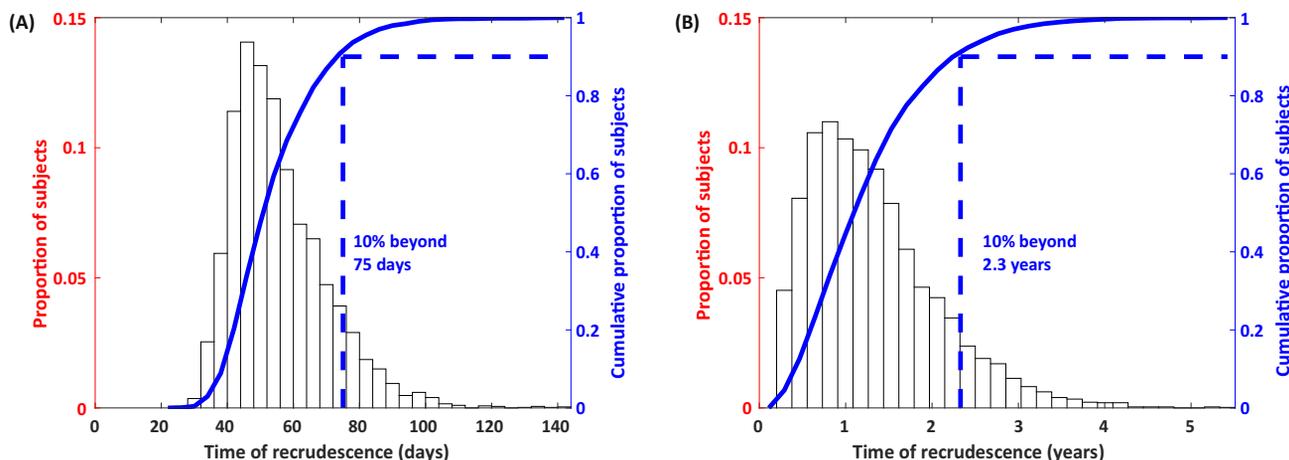


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**Figure 2. Hypothetical Infection Rates in Passive Broadly Neutralizing Anti-HIV Antibody (BnAb) Transfer Trial.** During BnAb therapy and while BnAb levels are maintained (shaded area), HIV infections are prevented or suppressed and the rate of detected new infections is lower in the BnAb group than with the placebo. If latent infections occur during BnAb therapy, when BnAb infusions cease an excess of infections due to recrudescence virus may be observed.

We could first assume that **reactivation rates** are similar to those of ART-suppressed chronic HIV infection (mean once per week [38]) and a BnAb infusion has fully suppressive immunity lasting 8 weeks [39] after which the lower BnAb levels are only partially effective. At partially suppressive levels of BnAb, some reactivations may still be successful but grow more slowly until BnAb levels wane further, with the reactivated virus potentially acquiring BnAb resistance. Under these conditions we predict that the median time to recrudescence infections will be 52 days after BnAb levels have decreased below their fully protective threshold. We find that 90% of all recrudescence infections will occur within 75 days after BnAb levels have dropped below this fully protective level (Figure 3A). Overall, in this highly dynamic reactivation model with the relatively rapid disappearance of the BnAb, most reactivated infections would be captured early after the BnAb is ceased (as in Figure 3A).

At the other end of the spectrum, however, is a scenario where reactivation events are rarer because of a very low number of founder viruses and the subtherapeutic tail of the BnAb is much longer, such as antibodies induced by vaccination. Reactivation of very low levels of latent infection (e.g., where viral DNA is not readily detected in blood) has been observed many months after ceasing ART in subjects undergoing bone marrow transplantation or in an infant treated very early after HIV acquisition [35,40]. The long duration before reactivation in these settings may in part reflect very low levels of latent infection, a scenario that we speculate may be akin to some cases of infection suppressed by vaccine-induced BnAbs. If vaccine-mediated BnAb generation is successful, the BnAbs may be durable for many months (as was observed for non-neutralizing antibodies in the RV144 trial) or many years (as is seen with other successful viral vaccines, such as recombinant hepatitis B vaccines). If we assume that successful reactivation rates are low (mean once per 6 months) and that the vaccine-induced **BnAb half-life of fully protective immunity** is 12 months, we model that the median time to detection of virus is 1.18 years after antibody levels have stopped being fully protective and that 90% of latent infections suppressed by the vaccine-induced BnAb would be detected by



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**Figure 3. Modeling Recrudescence of Latent HIV Infections Acquired Despite HIV-Specific Broadly Neutralizing Anti-HIV Antibodies (BnAbs).** The time of HIV recrudescence after BnAb levels begin to decline below fully protective levels is illustrated. Left-hand axes show histograms of the times at which subjects are expected to recrudescence. Right-hand axes show the cumulative proportions of subjects with infection. In (A) the model assumes that HIV reactivation occurs on average every 7 days (with a large pool of latent virus; e.g., chronic HIV infection) and the BnAb is fully protective for 8 weeks (e.g., a passively transferred antibody). In (B) the model assumes that HIV reactivation occurs on average every 6 months (e.g., with a very small pool of latent virus where HIV DNA is undetectable in blood) and the BnAb is fully protective for 1 year (e.g., as might occur after vaccination). Note that the x-axis measures years in model (B) and days in model (A). Details of the modeling are provided in the supplemental information online.

monitoring for 2.3 years after antibody levels dropped below the fully protective threshold (Figure 3B). If vaccine-induced BnAbs persist life long, any latent infections occurring despite the BnAb may never recrudescence. However, a situation where immunity wanes later in life during aging or under immunosuppressive therapy (analogous to tuberculosis or hepatitis B infection) may eventually lead to reactivated infection despite highly successful initial HIV immunity.

Modeling HIV reactivation in the context of waning vaccine-induced BnAbs is complicated by the likelihood of BnAb-inducing vaccines simultaneously eliciting non-neutralizing antibodies. These antibodies can mediate a wide array of non-neutralizing antibody functions, including ADCC, antibody-dependent phagocytosis, and antibody-dependent complement activation [41]. Arguably these antibodies should be able to contribute to the elimination of HIV-infected cells reactivating from latency. The utility of non-neutralizing antibodies, however, is dampened by their preferential recognition of CD4-induced epitopes on the viral envelope [22,23,42]. Downregulation of CD4 by the viral nef and vpu proteins decreases the availability of epitopes for many non-neutralizing antibodies. Epitope availability appears to be the main impediment to utilizing non-neutralizing antibodies to protect against HIV infection. Infection of humanized mice with a recombinant HIV reporter virus engineered to express influenza hemagglutinin (HA) is preventable with non-neutralizing antibodies directed towards HA [25]. Despite these caveats, some non-neutralizing antibodies bind to envelope fragments on infected cells and contribute to their elimination [25]. Thus, it is foreseeable that non-neutralizing antibodies induced by vaccination could contribute to holding at bay low-lying latent infections established on viral exposure. We hypothesize, however, that such immune responses might be less successful at restraining viral reactivation than BnAbs. Furthermore, non-neutralizing antibodies are susceptible to caveats similar to those of BnAbs. Non-neutralizing antibodies have been shown to drive viral escape [24,25]. Furthermore, antibodies associated with non-neutralizing functions, such as ADCC, wane following cessation of immunization [43].

These speculations about BnAb-suppressed latent HIV infections may also apply to CD8<sup>+</sup> T cell immunity to HIV. **'Exposed but uninfected' subjects** with HIV-specific T cell immunity have been described. These cases may reflect low-level latent infections initiated by allogeneic cells harboring HIV. One report described very low-level detection of HIV DNA in the blood of some such subjects [44]. Another report suggested that infrequent HIV exposure in Kenyan sex workers reduced levels of HIV-specific CD8<sup>+</sup> T cells and led to an increased rate of HIV infections [45]. This scenario could be explained by reactivation of earlier infections in the setting of reduced CD8<sup>+</sup> T cell immune surveillance, analogous to that described above for waning BnAb levels. An early macaque study suggested that cytotoxic T lymphocyte (CTL) recognition of allogeneic donor cells that share MHC I alleles with the host may provide better immunity [46].

### Concluding Remarks

In conclusion, we postulate that latent HIV infection suppressed by HIV-specific BnAbs can reactivate when immunity wanes. Cell-cell transmission could be one mechanism that evades BnAb induced control of infection. Recrudescence of latent infections controlled by BnAb and other immune responses should be explored in additional macaque and human vaccine and passive transfer efficacy trials. Depending on how long effective BnAb levels are maintained and the frequency of reactivation of HIV, this could require careful longer-term follow up. We suggest that, given the experience with ART, it should be no surprise that a retrovirus such as HIV could exist in a latent form controlled by immune responses. As we hopefully approach an era of HIV vaccine-induced protection, an open mind about the capacity for HIV to lie dormant and reactivate should be maintained.

### Supplemental Information

Supplemental information associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.it.2017.12.006>.

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### Outstanding Questions

Will recrudescence HIV infection after apparent protective immunity be a common occurrence in humans?

Where and with what frequency do latent infections occur despite the presence of BnAbs? Knowing the size and location of this reservoir of infection, and its frequency of reactivation and decay (or proliferation), will help guide efforts to study this field.

Defining recrudescence infections occurring late after BnAb therapy in humans may be difficult. A clear epidemiological and virological link would be helpful. Collection of such information could be built into such studies.

How important are Fc-mediated antibody functions in limiting latent infections occurring despite BnAbs? The study of BnAbs with Fc-mediated functions blocked in animal models may help answer this.

Will recrudescence HIV infections also be observed for partially successful CD8<sup>+</sup> T cell-based vaccines? Do 'exposed but uninfected' subjects represent a type of latent HIV despite T cell immunity?

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